510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111335

B. Purpose for Submission:

New assay

C. Measurand:

Adrenocorticotropic hormone

D. Type of Test:

Quantitative, Automated Fluorescence Immumoenzymatic Assay

E. Applicant:

Tosoh Corporation

F. Proprietary and Established Names:

ST AIA-PACK ACTH; ST AIA-PACK ACTH Calibrator Set; ST AIA-PACK ACTH Control

G. Regulatory Information:

1. Regulation section:

   862.1025 – Adrenocorticotropic hormone (ACTH) test system
   862.1150 – Calibrator
   862.1660 – Quality control material

2. Classification:

   Class II, II, and I, reserved

3. Product code:

   CKG, JIT, JJX

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use.

2. Indication(s) for use:

   ST AIA-PACK ACTH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Adrenocorticotropic hormone (ACTH) in human EDTA plasma on Tosoh AIA System Analyzers.

   Plasma ACTH measurements are useful in the differential diagnosis and treatment of certain disorders of the adrenal glands such as Cushing's syndrome, adrenocortical insufficiency, and ectopic ACTH syndrome.

   ST AIA-PACK ACTH Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK ACTH assay on Tosoh AIA Systems Analyzers.

   ST AIA-PACK ACTH Control Set is designed for IN VITRO DIAGNOSTIC USE ONLY for performing quality control procedures with the ST AIA-PACK ACTH assay on Tosoh AIA System Analyzers

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Tosoh AIA 2000 Analyzer.

I. Device Description:

Each kit contains 5 trays and 20 test cups (ST AIA-PACK ACTH Test Cup). (See principles of assay section for the specific components and their function it the test).

The following materials are also required to perform Adrenocorticotropic hormone analysis using the ST AIA-PACK ACTH on the TOSOH AIA System Analyzer. They are sold separately from the test cup by Tosoh, and are listed under "materials required but not provided". All performance data shown below was obtained using all these materials. Package inserts were included in the 510(k) for all these separately sold materials.

Substrate set II: 4-methylumbelliferyl phosphate, stabilizers and 0.01% sodium azide as a preservative (lyophilized).
Wash concentrate set - Buffer solution with detergent and bacteriostatic agent.

Diluent Concentrate Set - Buffer solution with detergent and 0.5% sodium azide as a preservative.

Diluting solution - BSA with no detectable ACTH plus preservative.

AIA-PACK ACTH Calibrator contains BSA with the assigned concentration of ACTH plus preservative. Target concentrations are:
0 pg/mL
15 pg/mL (approx.)
50 pg/mL (approx.)
300 pg/mL (approx.)
800 pg/mL (approx.)
2200 pg/mL (approx.)

AIA PACK ACTH Control Set contains BSA with approximately 50 pg/mL ACTH (level 1) and approximately 300 pg/mL ACTH (level 2).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Diagnostics Elecsys ACTH

2. Predicate 510(k) number(s):

   k060585

3. Comparison with predicate:

   The intended uses are the same. The technology is similar. A table comparing the two is shown below:

Item	Device	Predicate
	ST AIA-PACK ACTH	**Elecsys ACTH**
Intended Use/ Indications for use	Device is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Adrenocorticotropic hormone (ACTH) in human EDTA plasma on Tosoh AIA System Analyzers. ACTH measurements are useful in the differential diagnosis and treatment of certain disorders of the adrenal glands such as Cushing's syndrome, adrenocortical insufficiency, and ectopic ACTH syndrome.	Same
Intended Use- Calibrators	ST AIA-PACK ACTH Calibrator Set is intended for the calibration of the ACTH assay.	Same
Intended Use - Controls	The AIA-PACK ACTH CONTROL SET is intended for performing quality control procedures with the ACTH Assay.	Same
Assay Protocol	Sandwich assay	Same
Detection Method	Fluorescence	Electrochemiluminescent
Sample Type	Human EDTA Plasma	Same
Assay Low	2.0 pg/mL	1.0 pg/mL
Assay High	2000 pg/mL	2000 pg/mL
Reference Range observed in the sponsor's study	7.4-64.3 pg/mL	7.2-63.3 pg/mL
Number of control levels	Two levels of lyophilized control	Two levels of lyophilized control

Calibrator	ST AIA-PACK Calibrator Set 6-Point	ACTH CalSet 2-Point
Traceability / Standardization	ACTH (Human, 1-39) Bachem AG: code. H-1160; gravimetric preparation	Standardized gravimetrically with synthetic ACTH produced by Roche
Calibrator/Control Base Matrix	MOPSO Buffer with 5% Bovine Serum Albumin	Equine serum

K. Standard/Guidance Document Referenced (if applicable):

CLSI guidelines: C28-A3, Define and Determine Reference Intervals in the Clinical Laboratory; EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures; EP9-A2 Method Comparison and Bias Estimation Using Patient Samples; EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation.

L. Test Principle:

The ST AIA-PACK ACTH is a two-site enzyme immunoassay which is performed in the ST AIA-PACK ACTH test cups. ACTH present in the test sample is bound with goat polyclonal antibody immobilized on magnetic beads and enzyme-labeled polyclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled polyclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The enzyme alkaline phosphatase causes oxidation of 4MUP to 4MU. 4MU is excited at 365 nm and comes to ground state at 448 nm releasing fluorescent energy. The amount of fluorescent energy is measured by the detector. The amount of enzyme-labeled polyclonal antibody that binds to the beads is directly proportional to the ACTH concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

M. Performance Characteristics (if/when applicable):

Performance in this 510(k) was evaluated on the Tosoh AIA 2000 (Model 022100.)

1. Analytical performance:

a. Precision/Reproducibility:
The precision study followed the CLSI protocol, Evaluation of Precision Performance of Quantitative Measurement Methods (EP5-A2). The precision study for the ST AIA-PACK ACTH assay was evaluated utilizing three AIA-2000 analyzers and 3 different lots of reagents. Precision was assessed by assaying three levels of unaltered EDTA plasma specimens. Estimates of total and within-

run precision were obtained from measurements of 2 replicates in a single run, 2 times a day for 20 non-consecutive days. This equaled to a total of 40 runs and 80 determinants. One calibration curve (obtained on the first day) was used throughout.

The following results were obtained when tested with three unaltered plasma samples using three sets of reagents. A result of one reagent lot is shown below. Similar results were obtained with the two additional lots tested.

Within-run precision

Specimen	**Reagent Set # 1**		
	Mean (pg/mL)	**Pooled SD**	**CV %**
EDTA Plasma-A	37.8	1.2	3.1
EDTA Plasma-B	223.7	4.8	2.1
EDTA Plasma-C	709.2	10.9	1.5

Total precision:

Specimen	**Reagent Set # 1**		
	Mean (pg/mL)	**Pooled SD**	**CV %**
EDTA Plasma-A	37.8	1.2	3.3
EDTA Plasma-B	223.7	5.7	2.5
EDTA Plasma-C	709.2	15.6	2.2

Also see *Detection Limit* section below regarding precision at lower levels.

b. Linearity/assay reportable range:

The sponsor's claimed reportable range is 2-2000 pg/mL. To evaluate linearity, high and low samples (EDTA plasma) were serially diluted and assayed on the new device. The high samples were prepared by spiking a plasma sample and the low samples were prepared by diluting a low concentration sample with the AIA diluting solution. For each level tested, the average of four replicates was determined. The recoveries relative to expected concentrations based on dilution of the high and low samples, and the mean and SD's of the replicates, are listed below. (In additions see the Detection

Limit Section for additional recovery determinations near the claimed assay lower limits.)

Expected Value (pg/mL)	Observed Mean (n=4)	Percent observed/ expected	CV (%) (n=4)
1.9	1.9	100.0	6.2
38.1	35.9	94.2	2.7
74.3	74.8	100.6	1.9
146.7	152.0	103.6	2.5
243.2	242.2	99.6	1.9
485.6	481.6	99.2	1.0
725.9	738.3	101.7	1.5
967.3	983.3	101.7	2.7
1208.6	1234.5	102.1	2.1
1450.0	1440.2	99.3	0.7
1691.3	1696.9	100.3	1.6
1932.7	1946.7	100.7	2.6
2174.0	2145.2	98.7	2.0

The equation determined by linear regression was: Y=0.998X+4.5, R2=0.999.
In addition the data was evaluated according to CLSI guideline EP6 and the sponsor's criteria for linearity.

High dose hook was evaluated by spiking samples up to 1,000,000 pg/mL. The measured concentration read as > high limit, (although the raw signal decreased at concentrations > 100,000 pg/mL.

Dilutions of high spiked samples, 2000 to 20,000 starting concentrations. No significant bias (relative to expected) was observed.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Calibrators:
Value assignment and traceability:
The device is traceable to primary reference material containing commercially available purified ACTH (Human, 1-39), which is value assigned using another RIA test. Values were further validated by replicate measurements on multiple instruments for specific lots.

Calibrator values are as follows:

Target Values (pg/mL)	Estimated uncertainty in assigned values (pg/mL)[1]
0	0.1
15	1.7
50	5.4
300	31
800	81
2700	215

Real time storage:
Expiration of ST AIA-PACK ACTH CALIBRATOR SET was determined by using three different lots of CALIBRATOR SET, one AIA-2000 analyzer and three reagent sets. Reagents were stored at 8 - 12 degree C. The system was calibrated using the stored calibrator set on the day of assay. Each calibrator was assayed in 5 replicates and the mean and %CV were calculated, at multiple time points including and past the expiration date. The protocol and acceptance criteria provided were adequate for the claim of 12 months at 2-8 degrees C.

Calibrator in-use stability:
Three sets of calibrator material were opened and reconstituted at time points before the measurement, and stored refrigerated. The system was calibrated using the stored (test) calibrators and recovery and precision of pooled samples stored at -70 degree C were evaluated as unknowns. The protocol and acceptance criteria provided were adequate to support the claim of in-use calibrator stability of one day when stored for one day tightly sealed and refrigerated at 2-8 degrees C.

Control materials:
Stability:
Control materials opened and closed stability was evaluated as described for calibrators, except that open control stability testing extended to 7 days. The protocol and acceptance criteria were adequate to support shelf-life stability of months and open vial stability is 7 days when store at 2-8 degrees C.

Value assignment:
The AIA-PACK ACTH CONTROL SET contains assigned concentration ranges of ACTH. The assigned range is determined using two analyzers and two reagent lots with 5 replicates per sample using the manufacturer's working calibrators. The range is assigned as +/- 20% of the grand mean and is designed to provide target control levels of approximately 50 (target range approximately 40 to 60) and 300 (230 to 350) pg/mL of ACTH.

[1] relative to the commercial material and methods to which calibrators are traceable

d. Detection limit:

The lower limit of the assay range is based on the LoQ according to the CLSI EP17-A guideline. Fifteen samples were evaluated ranging from the LoB to 4 times the LoB. These samples were assayed 2 times a day for over 5 days. LoB is determined to be 0.5 pg/mL. LoD is determined by testing 60 blank and 60 low samples. LoD is determined to be 0.7 pg/mL. LoQ is determined to be 1.2 pg/mL. At that concentration CV was between 10- 20% (and bias based on linearity was < 5%).

The sponsor claimed that the measuring range of the assay is 2.0 to 2000 pg/mL

e. Analytical specificity:

ACTH fragments and related peptides were purchased from an outside vendor (Refer to the following table 1). ACTH fragments and related peptides were dissolved in ST AIA-PACK ACTH SAMPLE DILUTING SOLUTION (SDS) and adjusted to 10 ug/mL to prepare a stock solution. 30 uL of fragments were diluted with 2,970 uL of EDTA plasma to 100,000 pg/mL. Concentrations of 500 and 5,000 pg/mL were prepared by further dilution with EDTA plasma.

The specimens were measured in duplicate using the ST AIA-PACK ACTH assay at one site using one AIA-2000 analyzer and a single lot of reagent each. The mean and percent recovery was calculated as (Apparent value / Control value) x 100.

ACTH Fragment	ACTH fragment conc. [pg/mL]	Average value	Difference (test- – control)	ACTH Recovery [%]	% Cross-reactivity
(Control – without fragmets)	0	54.3	---	---	---
ACTH 1-10	500	54.3	0.0	100%	-0.01%
	5,000	54.7	0.4	101%	0.01%
	100,000	55.7	1.3	102%	0.00%
ACTH 11-24	500	53.4	-0.9	98%	-0.18%
	5,000	53.6	-0.8	99%	-0.02%
	100,000	53.8	-0.5	99%	0.00%
Beta-MSH	500	53.8	-0.5	99%	-0.10%
	5,000	54.5	0.2	100%	0.00%
	100,000	51.6	-2.8	95%	0.00%

Beta-Endorphin	500	53.0	-1.3	98%	-0.26%
	5,000	54.2	-0.1	100%	0.00%
	100,000	52.8	-1.5	97%	0.00%

ACTH Fragment	**ACTH fragment conc. [pg/mL]**	**Average Measured ACTH conc. [pg/mL]**	**Difference (test - control)**	**ACTH Recovery [%]**	**% Cross-reactivity**
(Control)	0	54.3	---	---	---
ACTH 1-17	500	50.1	-4.2	92%	-0.85%
	5,000	25.6	-28.8	47%	-0.58%
	100,000	2.0	-52.3	4%	-0.05%
ACTH 1-24	500	49.6	-4.7	91%	-0.94%
	5,000	25.4	-29.0	47%	-0.58%
	100,000	2.0	-52.4	4%	-0.05%
ACTH 18-39	500	53.3	-1.1	98%	-0.21%
	5,000	39.0	-15.3	72%	-0.31%
	100,000	3.5	-50.8	6%	-0.05%
ACTH 22-39	500	52.8	-1.6	97%	-0.32%
	5,000	50.1	-4.2	92%	-0.08%
	100,000	14.6	-39.7	27%	-0.04%
alpha-MSH	500	49.7	-4.7	91%	-0.93%
	5,000	25.6	-28.7	47%	-0.57%
	100,000	10.3	-44.1	19%	-0.04%

ACTH 1-10, 11-24, beta-MSH and beta-Endorphin do not interfere the assay up to 100,000 pg/mL.
ACTH recovery was less than 90% in the presence of ACTH 1-17, 1-24, 18-39, alpha-MSH (>5,000pg/mL) or ACTH 22-39 (>100,000 pg/mL). These fragments may negatively affect the ACTH assay. It is likely that these fragments bind to either the antibodies on beads or the enzyme-labeled antibodies. Therefore, if these fragments were contained excessively in the test sample, lower values may be reported.

Evaluation of Common exogenous compounds:
Specimen pools at three concentrations of ACTH were evaluated by adding interfering substance and assaying utilizing the ST AIA-PACK ACTH assay on the AIA-2000. The average of triplicate measurements for each level was calculated. The specimen value without adding interfering substance was used as the reference value. The sponsor defines non-significant interference as recovery within± 10% of the unspiked samples. No significant interference was observed when endogenous compounds were tested up to the following concentrations.

Heparin (up to 50 U/mL).
Acetominophen (up to 20 mg/L).
Acetylsalicylic acid (up to 300 mg/L).
Ampicillin (up to 200 mg/L).
Ibuprofen (up to 50 mg/L).
Theophylline (up to 10 mg/L).
Hemoglobin (up to 440 mg/dL)
Conjugated bilirubin (up to 19 mg/dL)
Free bilirubin (up to 19 mg/dL)
Lipemia (up to 1667 mg/dL)
Rheumatoid factor (up to 500 IU/mL)

2. Comparison studies:

a. Method comparison with predicate device:
A total of 160 EDTA plasma specimens were assayed in singleton utilizing the ST AIA-PACK ACTH assay on the AIA-2000 analyzer and the Roche Elecsys-ACTH on the MODULAR ANALYTICS E170 (Elecsys module) analyzer. A combination of fresh and frozen specimens was utilized for this study. Sample selection criteria included only sample stability and integrity (e.g., no known hemolysis, lipemia or icteric). Of the 160 samples.154 were unaltered patient samples. Another 6 were prepared by mixing patient samples. Samples exposed to repeated freeze-thaw cycles were not used. Samples containing particulate matter were centrifuged prior to testing. Prior to assay, frozen samples were brought to room temperature slowly, and mixed gently.

Testing was done at one site utilizing one AIA-2000 analyzers and one MODULAR ANALYTICS E170 (Elecsys module) analyzer. Results are shown below.

Regression Analysis		
	Deming	**Linear regression**
Slope:	1.10 (1.075 to 1.116)	1.09 (1.067 to 1.107)
Intercept:	-0.84 (-8.921 to 7.234)	0.76 (-7.305 to 8.817)
95% Confidence Intervals are shown in parentheses		
Corr Coef (R):	0.993	

Points (Plotted/Total):	160/160
Result Ranges:	3.8 to 1986 pg/mL

b. Matrix comparison

Not applicable; specimen collection and handling states EDTA plasma is required for the assay.

3. Clinical studies:

   a. Clinical Sensitivity: Not applicable; Clinical sensitivity and specificity is not typically provided in 510(k)s for this type of assay.

   b. Clinical specificity: See a, above.

   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable

4. Clinical cut-off:

   Not applicable; this is a quantitative assay.

5. Expected values/Reference range:

   The package insert states that each laboratory should determine a reference interval corresponding to the characteristics of the population being tested. Clinical results must be interpreted with regard to concomitant medications administered to the patient.

   The reference range study was conducted with reference to the CLSI protocol entitled: How to Define and Determine Reference Intervals in the Clinical Laboratory (C28-A3).
   A total of 121 well-characterized unaltered EDTA plasma specimens from ambulatory patients with no known history of pituitary or adrenal disease were assayed in singleton utilizing the ST AIA-PACK ACTH assay on the AIA-2000 analyzer. Specimens from 110 males between the ages of 26 to 60 years and 11 females between the ages of 28 to 44 years were included in this study. Samples were collected between 8 AM and noon and frozen below -70 degree C until testing. Prior to assay frozen samples were brought to room temperature (18 - 25 °C) slowly, and mixed gently. Samples exposed to repeated freeze-thaw cycles were not used. Samples containing particulate matter were centrifuged prior to testing.

   Testing was done at one site utilizing one AIA-2000 analyzer. Instrument maintenance and QC records were reviewed to ensure the instrument was functioning within normal guidelines.

Central 95% Interval		
N = 121		
Lower Reference Limit 5% range		**Upper Reference Limit 95% range**
Nonparametric	7.4 [6.0 to 8.9] pg/mL	64.3 [48.4 to 72.4] pg/mL

N. Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.